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Certain observations on the olfactory pathway

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INTRODUCTION

A considerable amount of work has been done on the olfactory pathways in recent years, and although there is now general agreement as to the distribution of the major fibre projections from the olfactory bulb, certain discrepancies require clarification. One of the most prominent of these is whether the olfactory bulb and/or the pyriform cortex project to the entorhinal area. Le Gros Clark & Meyer (1947) and Cragg (1961) in the rabbit, Meyer & Allison (1949) in the monkey, and Powell, Cowan & Raisman (1965) in the rat have indicated that the fibres from the olfactory bulb do not extend caudal to the pyriform cortex, although both Cragg (1961) and Powell *et al.* (1965) have reported that the pyriform cortex itself projects caudally into the entorhinal area. On the other hand, White (1965) and Heimer (1968) in the rat and Scalia (1966) in the rabbit have reported that the olfactory bulb projects directly to the ventrolateral part of the entorhinal cortex. A second problem is whether the entire extent of the olfactory tubercle receives an olfactory projection, as is suggested by the work of White (1965) and Heimer (1968) or whether the efferent fibres from the olfactory bulb are restricted in their termination to the antero-lateral part of the tubercle, as is indicated by the reports of most other authors (Le Gros Clark & Meyer, 1947; Meyer & Allison, 1949; Cragg, 1961; Lohman, 1963; Powell, *et al.* 1965; Scalia, 1966). Thirdly, Scalia (1966) has reported that the olfactory bulb projects to the anterior hippocampus in the rabbit, a finding which has not been reported by any other worker using experimental methods.

There are several possible explanations for these discrepancies. The reduced silver methods for staining degenerating axons are notoriously capricious, and it is possible that some workers may have been able to produce more complete impregnation of their sections and as a consequence have seen projections which others have missed. Also, in the olfactory system, as in other parts of the brain, it is often difficult to ensure that a supposedly localized lesion has not extended into other areas (e.g. from the olfactory bulb into the anterior olfactory nucleus) or that it has not interrupted fibres of passage. Finally, the olfactory pathways may be topographically organized in such a manner that some authors, especially those using small lesions, may have seen only a portion of a given pathway (e.g. from the olfactory bulb to the olfactory tubercle).

In the present study an attempt has been made to resolve the three problems, and in the course of it observations have also been made (1) on a direct projection from

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the pyriform cortex into the stria medullaris, (2) on the organization of the projection from the anterior olfactory nucleus of one side through the anterior commissure to the anterior olfactory nucleus of the opposite side, and (3) on a pathway to the anterior hippocampus from the more caudal portions of the rhinencephalon.

MATERIALS AND METHODS

Observations were made on the brains of 32 rats. Nineteen of these, with lesions in the pyriform cortex and amygdala, were also used in previous papers (Price & Powell, 1970*a*, *b*), but in addition seven brains with lesions in the olfactory bulb, three brains with lesions in the olfactory bulb together with damage to the anterior olfactory nucleus, and three brains with lesions in the entorhinal cortex were prepared for this study. The lesions were placed under direct vision, and the animals were allowed to survive for 4 days. In order to study different parts of the brain to best advantage, the brains were sectioned on a freezing microtome in either the frontal, sagittal or horizontal plane, depending on the site of the lesion in each brain. A 1 in 10 series was stained in the first instance, using the method of Nauta & Gygax (1954), or its recent modifications by Fink & Heimer (1967), and Wiitanen (1969). In many cases the series was counterstained with cresyl violet, or an alternate series was mounted and stained with thionin.

The criteria for identifying degenerating fibres and terminals have been discussed in previous papers (Price & Powell, 1970*a*, *b*). The different staining procedures all gave the same pattern of distribution of the degeneration, although the more recent methods (Fink-Heimer or Wiitanen) were generally easier to use and interpret.

RESULTS

Lesions of the olfactory bulb

Small lesions were made in the olfactory bulb which were unquestionably restricted to the bulb and did not damage the anterior olfactory nucleus. One of the experiments (R 761, Fig. 1) will be described in detail; the brain was cut sagittally and alternate series were stained with the Nauta–Gygax and Fink–Heimer methods, in addition to which a series was stained with thionin. The lesion destroyed most of the dorsolateral surface of the bulb, extending through the external plexiform layer into the underlying granule cell layer. The pars rostralis of the anterior olfactory nucleus was not encroached upon, and this was confirmed by the absence of degeneration in the anterior commissure and the contralateral bulb, for several investigators (Lohman, 1963; Powell *et al.* 1965; White, 1965; Heimer, 1968) have shown that degenerating fibres are not found in the anterior commissure unless the anterior olfactory nucleus has been damaged.

Within the superficial layers of the olfactory bulb of this brain degenerating fibres can be seen to extend for considerable distances from the lesion in the periglomerular region and in the external plexiform layer. In the internal plexiform layer immediately deep to the mitral cell layer, a particularly high concentration of degenerating fragments is found throughout all parts of the main bulb. This fragmentation in the internal plexiform layer is presumably due mainly to the axon collaterals of the

mitral and tufted cells which are known to ramify extensively in this layer (Cajal, 1911). All layers of the accessory olfactory bulb are clear of degeneration, except for the tract of myelinated fibres passing between the external plexiform and granule cell layers of the accessory bulb; this tract contains many coarse fragments of degenerating fibres.

The degenerating efferent axons from the olfactory bulb pass through the granule cell layer and into the lateral olfactory tract. In the olfactory peduncle, terminal degeneration is largely but not completely restricted to the superficial half of the plexiform layer of the anterior olfactory nucleus (Heimer, 1968); this is especially obvious in the pars lateralis and the pars dorsalis. A few degenerating fibres and possibly degenerating terminals are also found in the granular layer deep to the pyramidal cell layer.

ABBREVIATIONS

AC	anterior commissure	*Glom*	glomerular layer of olfactory bulb
AH	anterior hippocampus	*H*	hippocampus
AM	amygdaloid nuclei	*LOT*	lateral olfactory tract
AON	anterior olfactory nucleus	*OB*	olfactory bulb
ENT	entorhinal area proper (spheno-occipital ganglion of Cajal)	*OT*	olfactory tubercle
		PC	pyriform cortex
EPL	external plexiform layer of olfactory bulb	*S*	corpus striatum
GCL	granule cell layer of olfactory bulb	*SM*	stria medullaris

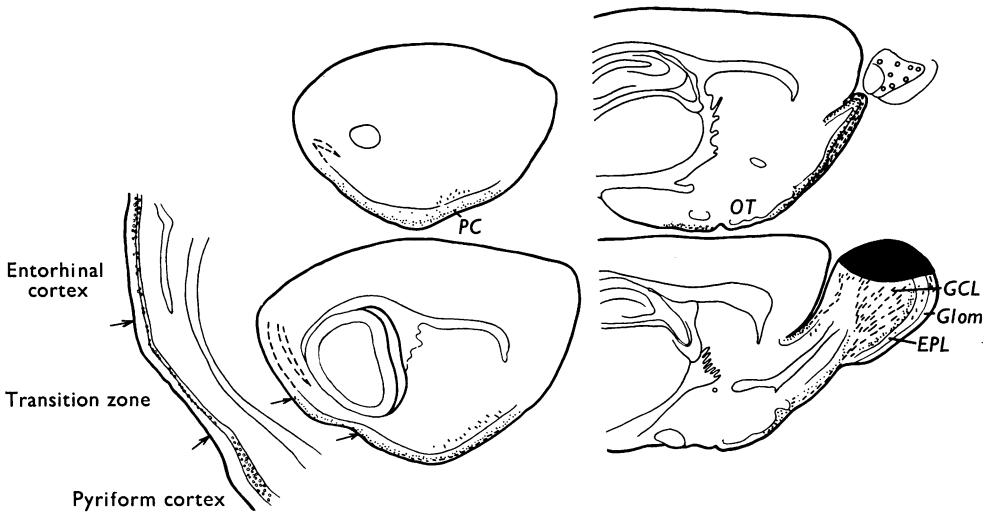


Fig. 1. The lesion and the degeneration in R 761, together with a tracing taken from an alternate section, stained with thionin, of the transition region between the spheno-occipital ganglion of Cajal and the pyriform cortex. Dashes indicate fibre degeneration, and dots terminal degeneration.

Degenerating fibres leave the medial side of the lateral olfactory tract in R 761 to pass into the antero-lateral part of the olfactory tubercle, where terminal degeneration is restricted to the superficial two-thirds of the plexiform layer. The postero-medial part of the tubercle is virtually clear, although there is well-stained degeneration in the amygdala immediately caudal to it. This limited projection to

the olfactory tubercle can be seen over many sections, and is apparent with the Fink–Heimer as well as the Nauta–Gygax method.

Because of the disagreement in the literature as to whether or not the olfactory bulb projects to the postero-medial portion of the olfactory tubercle, the results of lesions placed in various parts of the olfactory bulb in four additional rats (Fig. 2) will be described. Two of these lesions were placed in the dorsal part of the olfactory bulb (R 9 and R 10), while the other two were placed in the ventral part of the bulb (R 82 and R 83). In order to determine the extent of the lesions, the olfactory bulbs were removed and cut sagittally, while the rest of the brains were cut frontally. Careful examination of these sections shows that the damage does not extend into the anterior olfactory nucleus. In all these brains there is well-stained, heavy degeneration of both fibres and terminals in the antero-lateral part of the olfactory

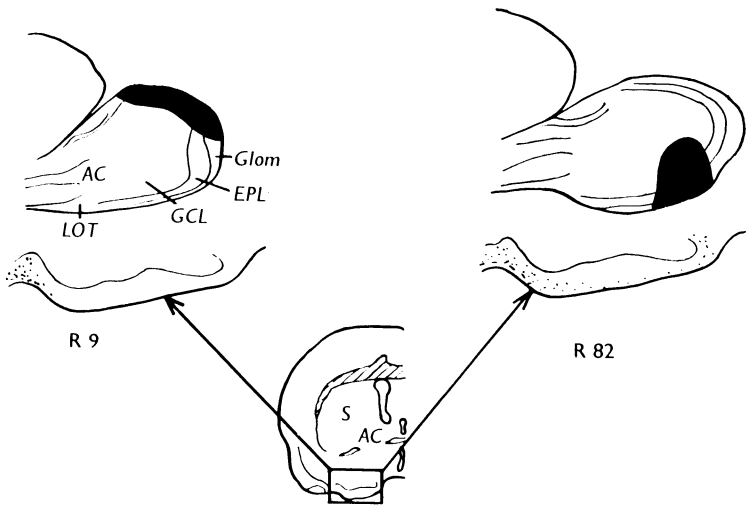


Fig. 2. The lesions in the olfactory bulb and the resultant degeneration in the most caudal section of the olfactory tubercle in R 9 and in R 82. In R 9, with a dorsal lesion, the degeneration is only in the antero-lateral part of the tubercle, whereas in R 82, with a ventral lesion, degeneration is present throughout the olfactory tubercle.

tubercle. In the two brains with lesions in the dorsal part of the olfactory bulb there is a clear line of demarcation between the antero-lateral part of the tubercle and the postero-medial part, which contains little or no sign of any degeneration (Fig. 4). On the other hand, in the two brains with lesions in the ventral part of the bulb, there is no clear line between the two parts of the olfactory tubercle, and there is definite evidence of terminal degeneration throughout the whole extent of the tubercle, including its most medial and posterior parts (Fig. 3). This degeneration is sparser and somewhat more patchy than that in the antero-lateral part of the olfactory tubercle, and while the difference in the density of degeneration in the two regions may be due simply to the fact that the degenerating fibres must pass through the antero-lateral part of the tubercle before reaching its postero-medial part, it could be due to the small size of the ventral lesions. These brains clearly indicate that the

ventral part of the olfactory bulb, but presumably not the dorsal part, projects to the postero-medial portion of the olfactory tubercle.

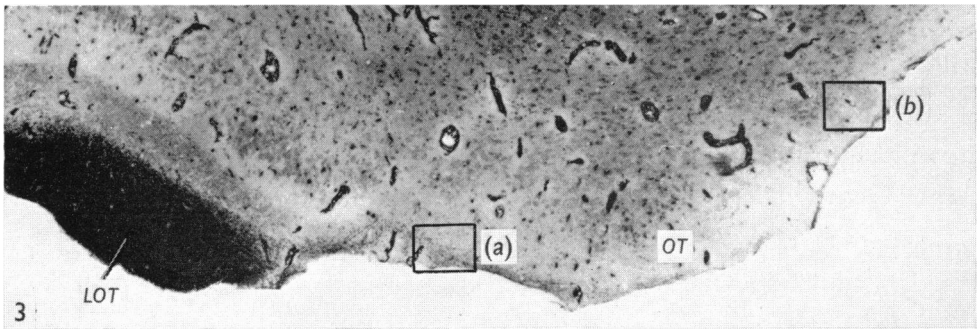
The anterior rudiment of the hippocampus cannot be studied in the sagittal sections of R 761, but it can be seen in the four brains described above, which were sectioned frontally (R 9, R 10, R 82, R 83). In none of these brains is there either fibre or terminal degeneration in or superficial to the anterior hippocampus, although in the same sections there is well-stained degeneration in the pyriform cortex and in the olfactory tubercle.

A laminar pattern of degeneration similar to that in the anterior olfactory nucleus is also found in the pyriform cortex of all brains with lesions of the olfactory bulb. We define the term pyriform cortex as all that cortex from the anterior olfactory nucleus to the entorhinal cortex, deep and lateral to the lateral olfactory tract, lateral to the amygdala, and medial to the rhinal sulcus; included within the pyriform cortex is therefore the 'periamygdaloid cortex' of some other authors (e.g. Lohman, 1963). Terminal degeneration is found principally in the superficial half of the plexiform layer, where it forms a clear, well-defined band. Although the deep boundary of this band is quite sharp, especially in the rostral parts of the pyriform cortex, scattered silver granules indicative of degenerating terminals are found in the deeper half of the plexiform layer, and there are degenerating fibres (and probably terminals) among the cells deep to the superficial pyramidal cells. These deeper fibres appear to have passed deeply from the surface through the superficial pyramidal cells, and are not derived from the deeper fibre pathway which has been described as associated with the anterior commissure, and which may arise in the anterior olfactory nucleus (Cragg, 1961; Lohman, 1963; Powell *et al.* 1965).

No difference was found in the distribution of the degeneration in the pyriform cortex between the brains with lesions in the dorsal part of the olfactory bulb (R 9 and R 10) and the brains with lesions in the ventral part of the bulb (R 82 and R 83). No evidence, therefore, was obtained which would indicate that there is an organized topographical projection from the olfactory bulb on to the pyriform cortex.

The distribution of degenerating fibres to the amygdala is limited to the molecular layer overlying the nucleus of the lateral olfactory tract and the cortical nucleus. Little, if any, degeneration is seen in the more caudal portions of the cortical nucleus or in the medial nucleus except after larger lesions extending into the accessory olfactory bulb and the anterior olfactory nucleus, and no degeneration is ever found in other parts of the amygdala after lesions of the olfactory bulb or peduncle. These observations are compatible with the recent report by Winans & Scalia (1970) that only the accessory olfactory bulb projects to the caudomedial segment of the cortical amygdaloid nucleus.

In the sagittally cut brain of R 761 there is also terminal degeneration in the superficial molecular layer of the cortex caudal to the pyriform cortex; this degeneration is continuous with the degeneration in the pyriform cortex, but it extends up on to the caudal surface of the hemisphere, around the ventral pole of the hippocampus. The fibre degeneration which is present in the most superficial part of the pyriform cortex diminishes near the posterior edge of the pyriform cortex, however, and only fine granular degeneration is found beyond that point (Fig. 11). This fine granular degeneration is most easily identified in sections stained with the Fink–Heimer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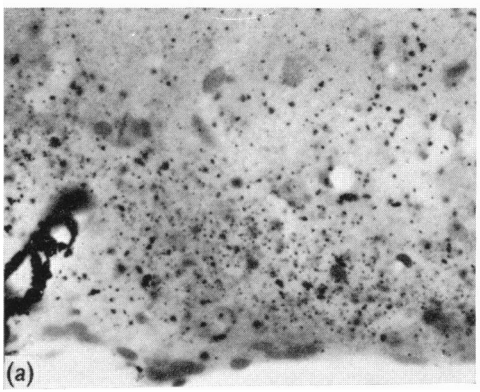
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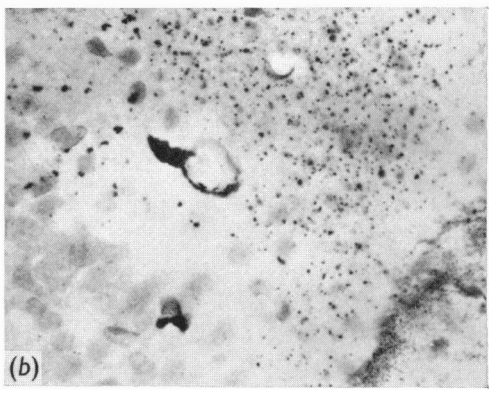
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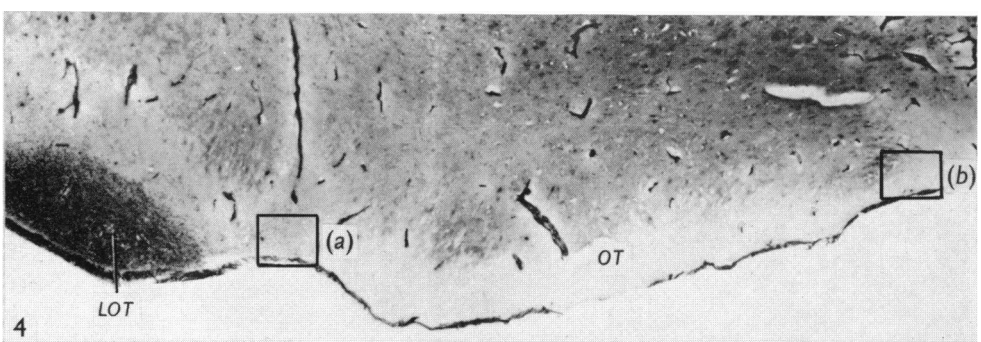
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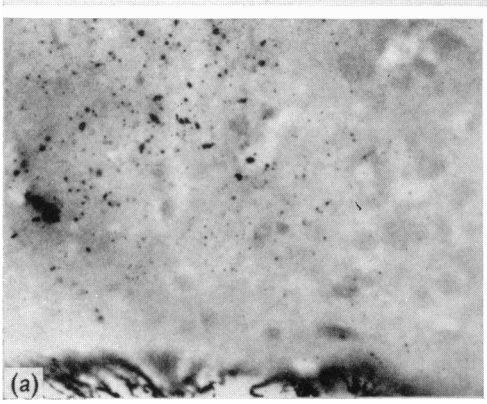
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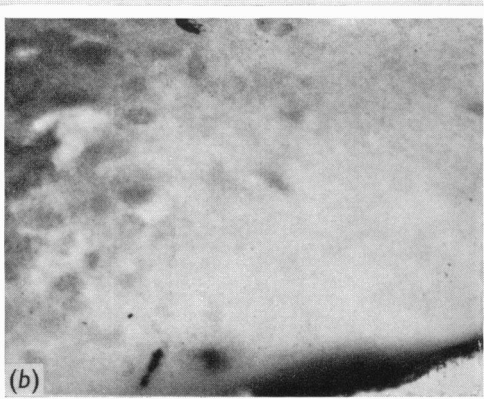
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method, but it is also shown by the Nauta–Gygax method; the distribution and extent of the degeneration is the same with both techniques. Degeneration caudal to the pyriform cortex is present in all the brains with lesions of the olfactory bulb, but, due to the curvature of the rat brain in this region, the precise distribution of the degeneration can only be determined in sagittal sections.

Although the cortex caudal to the pyriform cortex is generally recognized as the entorhinal cortex, the precise definition of this area has varied somewhat from one author to another. In order to define the precise limits of the degeneration seen after lesions of the olfactory bulb, it is necessary to give a brief description of the region and of the architectonic distinctions between different parts of it. The entorhinal area of Rose (1929–1931) and others is somewhat larger than the area originally described by Cajal (1911) as the 'angular' or 'spheno-occipital' ganglion (see Blackstad, 1956; Scalia, 1966); anterior and ventral to this 'ganglion' is Cajal's sphenoidal cortex, which he considered to receive fibres from the olfactory bulb. From the description and illustrations of Cajal (Fig. 5) the spheno-occipital ganglion can be readily recognized by the presence of a well-marked deep plexiform or IVth layer (see, for example, Cajal, 1911, vol. II, fig. 451). In order to determine precisely the relation between the degeneration and the architectonic structure of this region, an alternate series of sections from R 761 was mounted and stained with thionin. A correlation of the extent of the degeneration in the experimental material with the thionin-stained sections shows that the degeneration stops sharply at the ventral margin of the cortex with a deep plexiform layer and therefore does not enter the spheno-occipital ganglion as described by Cajal.

In Nissl material (Fig. 6) the part of the entorhinal region which receives the olfactory projection appears transitional in structure between the pyriform cortex and the spheno-occipital ganglion. The major changes in structure between the spheno-occipital ganglion and the transition region are found in the middle layers of the cortex. In the spheno-occipital ganglion the deep plexiform layer is bounded superficially by a well-defined zone of medium pyramidal cells, and deeply by a layer of small pyramidal cells. (The fifth layer of Cajal, between the deep plexiform layer and the layer of small pyramidal cells, consists of horizontal fusiform cells and is narrow and difficult to distinguish in material stained by the Nissl method). In the transition zone these three layers merge, and the deep plexiform layer cannot be distinguished. On the other hand, between the transition region and the pyriform cortex, the major difference is in the second layer. In the pyriform cortex this layer is thicker and composed of larger cells, while in the transition region its most superficial cells are smaller and are often arranged into small clumps or islands, similar to those in parts of the entorhinal cortex proper.

Fig. 3. The most caudal section through the olfactory tubercle in R 82, which had a lesion in the ventral portion of the olfactory bulb (× 56). (*a*) High-power view of the lateral part of the olfactory tubercle in the same section, showing terminal degeneration (× 480). (*b*) The most medial part of the tubercle, again showing terminal degeneration (× 480).

Fig. 4. The most caudal section through the olfactory tubercle in R 9, which had a lesion in the dorsal part of the olfactory bulb (× 56). (*a*) The most lateral part of the olfactory tubercle in the same section, showing the medial limit of the terminal degeneration (× 480). (*b*) The most medial part of the olfactory tubercle, which is completely free of terminal degeneration (× 480).

Lesions involving the anterior olfactory nucleus

A systematic description of experiments in which lesions of the olfactory bulb extended into the anterior olfactory nucleus will not be given as our results are in general agreement with those of other workers (Lohman, 1963; Powell *et al.* 1965),

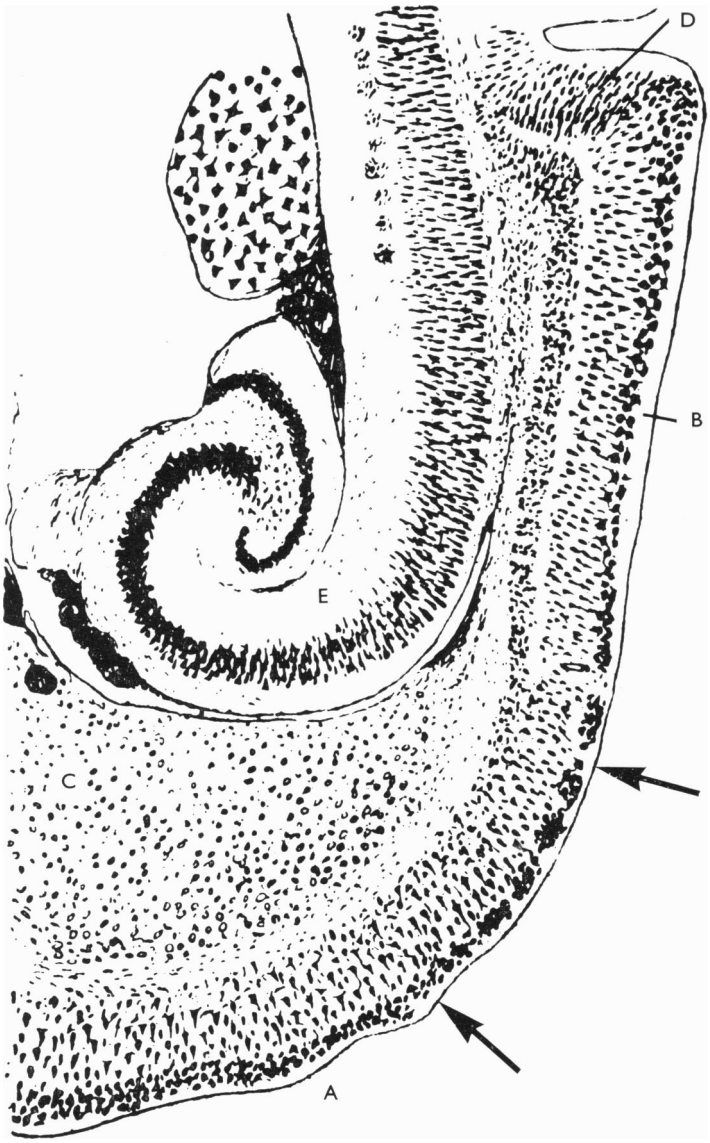


Fig. 5. Reproduction of fig. 451 (volume II) of Cajal (1911) to show his interpretation of the structure and limits of the 'true olfactory sphenoidal cortex' (A) and of the 'spheno-occipital ganglion' (B) in a 'central sagittal section of the pyriform lobe of the cat'. D is the superior limit of the spheno-occipital ganglion, E is the Ammon's Horn and C the lenticular nucleus. We have inserted the arrows to indicate our interpretation of the presence and site of the transition zone, although it was not identified as such by Cajal.

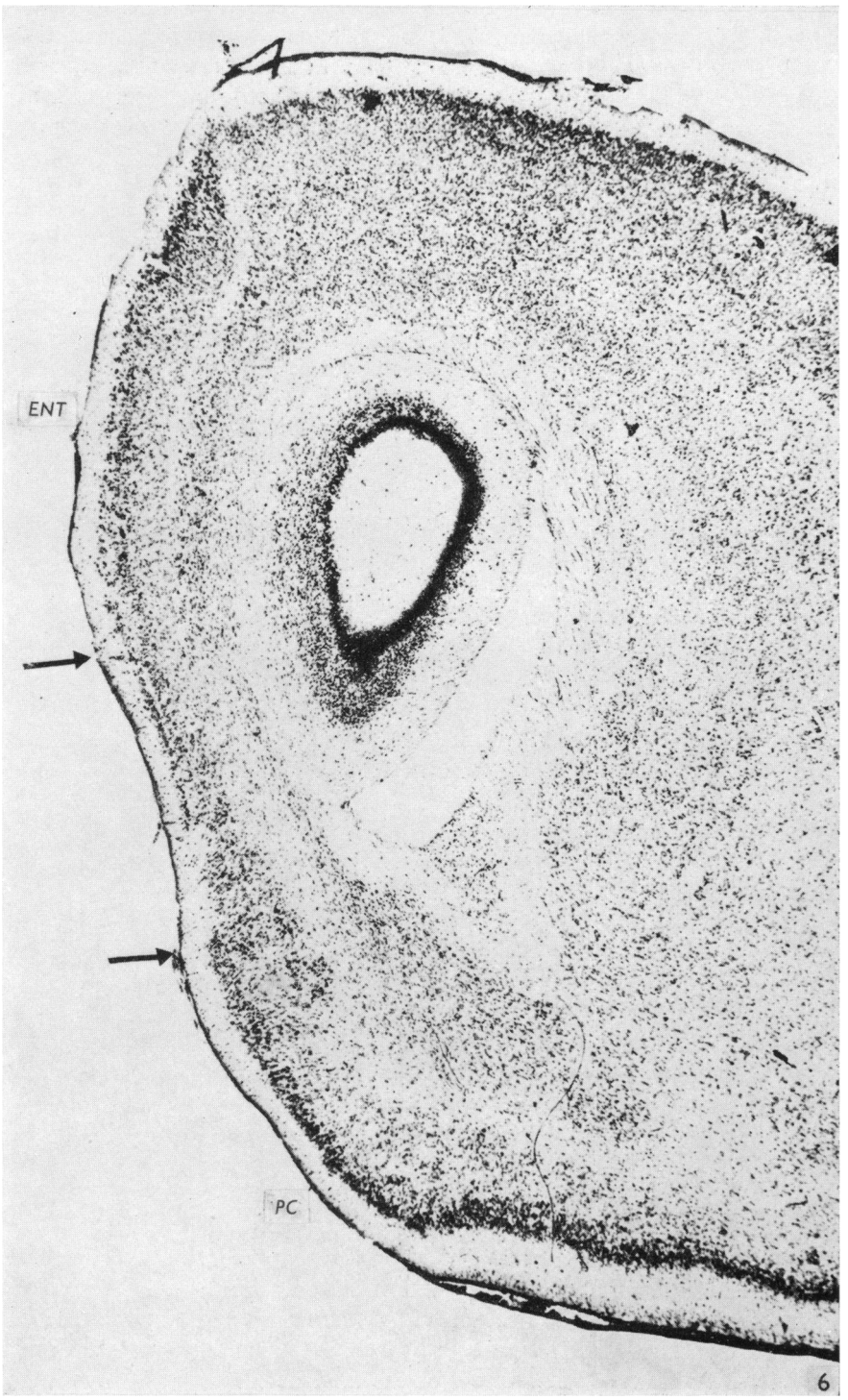


Fig. 6. Photomicrograph of sagittal section to show the spheno-occipital ganglion of Cajal or the entorhinal cortex proper (*ENT*), the pyriform cortex (*PC*) and the region of transition between them (between the arrows). Note the characteristic deep plexiform layer in the entorhinal cortex and the thickened, second layer of cells in the pyriform cortex. (Thionin, × 16.)

but two points may be made. The first of these is that in the brains with damage to the anterior olfactory nucleus (e.g. R 764, Fig. 7) there is heavy degeneration through the full extent of the olfactory tubercle. However, in these brains the entire olfactory bulb as well as a substantial portion of the anterior olfactory nucleus was destroyed. The second point relates to the distribution of terminal degeneration in the anterior olfactory nucleus of the contralateral side. After the large lesion in R 764, the degeneration is concentrated in the deep half of the plexiform layer of the nucleus

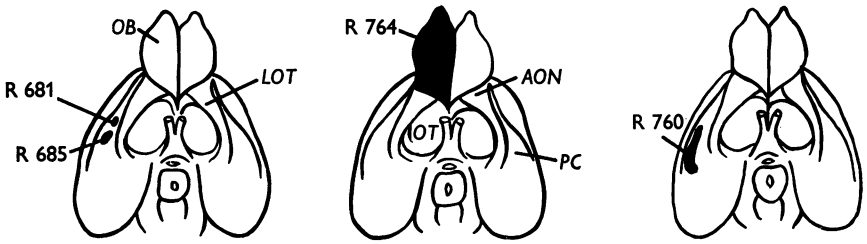


Fig. 7. Surface reconstructions of the lesions in R 681 and R 685, which were cut frontally, and in R 760 and R 764, which were cut sagittally.

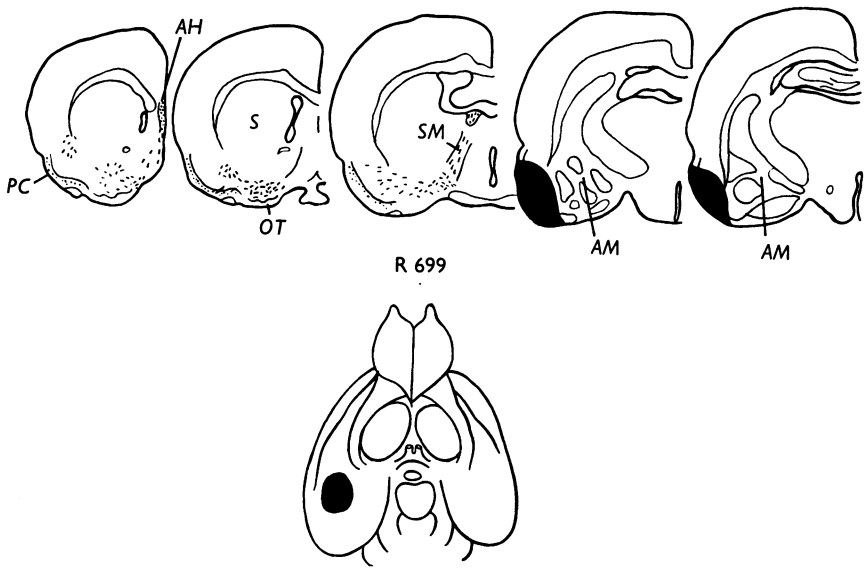


Fig. 8. The lesion and the resultant degeneration passing into the stria medullaris and the anterior hippocampus in R 699.

and is therefore complementary to the degeneration present in the superficial half of the plexiform layer after a lesion of the ipsilateral olfactory bulb. Furthermore, the termination of the commissural fibres is predominantly in the ventro-lateral part of the anterior olfactory nucleus, deep to the lateral olfactory tract, with relatively little degeneration in the dorsal aspect of the nucleus (Fig. 14). This is in contrast

to the degeneration found after a lesion of the pyriform cortex, which is predominantly in the dorsal portions of the nucleus (Price & Powell, 1970*a*).

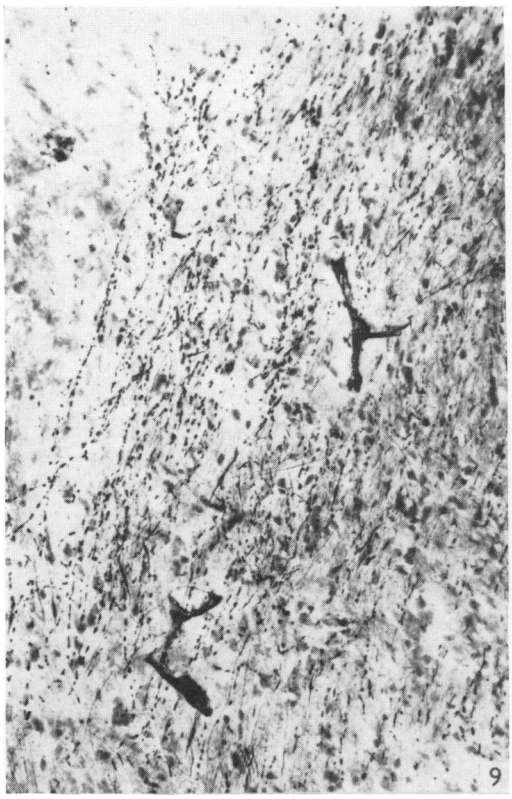
It should also be mentioned that the distribution of the degeneration in the entorhinal area is the same as that found after the relatively small olfactory bulb lesion in R 761, although the density of the degeneration is greater.

Lesions of the pyriform cortex

Our results on the projections of the pyriform cortex are also in good agreement with those reported previously (Powell *et al.* 1965; Heimer, 1968; Price & Powell, 1970*a*) and only certain points need be considered here. These relate to a projection to the lateral habenular nucleus through the stria medullaris and the projection into the entorhinal area.

In all brains with lesions of the pyriform cortex which have been studied, in addition to the projection through the thalamic radiation to the medio-dorsal thalamic nucleus and to the lateral habenular nucleus (Powell *et al.* 1965), a variable number of degenerating fibres enters the stria medullaris from the medial forebrain bundle and then passes through the stria to the lateral habenular nucleus, which is filled with degenerating fragments. None of these fibres crosses in the habenular commissure to the opposite side. This projection is best seen in brains such as R 699 (Fig. 8), in which a large lesion of the posterior pyriform cortex destroyed a considerable portion of this cortical area; in this brain the degenerating fibres are relatively numerous and make up a substantial fraction of the lateral part of the stria medullaris (Figs. 9, 10). However, even in brains such as R 685 and R 681 (Fig. 7) with small lesions of the anterior pyriform cortex, in which only a small area of the cortex was damaged, a few degenerating fibres can always be seen in the stria, particularly when the normal fibres have been well suppressed. This component of the stria medullaris would appear therefore to arise from the entire pyriform cortex, and the degree to which it degenerates in any individual experiment varies directly with the extent of that cortex which was damaged; it is probable that the proportion of the stria medullaris which is composed of this direct projection from the pyriform cortex is quite substantial. Although the more anterior lesions would not have interrupted fibres from any part of the brain except the pyriform cortex, the posterior lesions might have interrupted fibres from the amygdala, and an additional projection from this complex into the stria medullaris cannot be excluded.

Following lesions of the pyriform cortex, degeneration can also be seen extending caudally into the entorhinal region; in sagittally cut brains (e.g. R 760, Fig. 7) this degeneration is found to have precisely the same distribution as that found after a lesion of the olfactory bulb, stopping sharply at the border of the spheno-occipital ganglion. The degeneration is most marked after damage to the posterior pyriform cortex, and it grows progressively sparser and less distinct with more anterior lesions. Because the fibres from the olfactory bulb travel through the fibrillar layer on the surface of the pyriform cortex to reach the entorhinal region they will be interrupted by any lesion of the pyriform cortex, particularly in its more posterior parts, and it is possible that the degeneration seen in these experiments is due to involvement of the fibres from the olfactory bulb. An additional projection to the entorhinal area from the pyriform cortex cannot be excluded.



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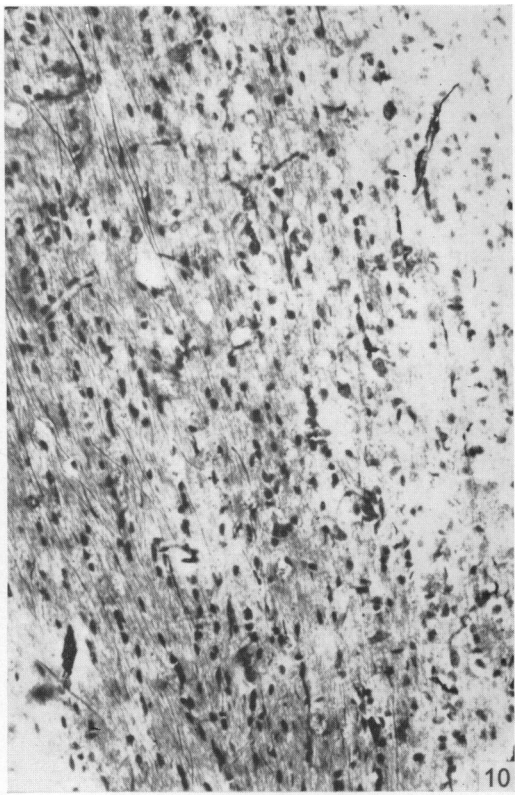

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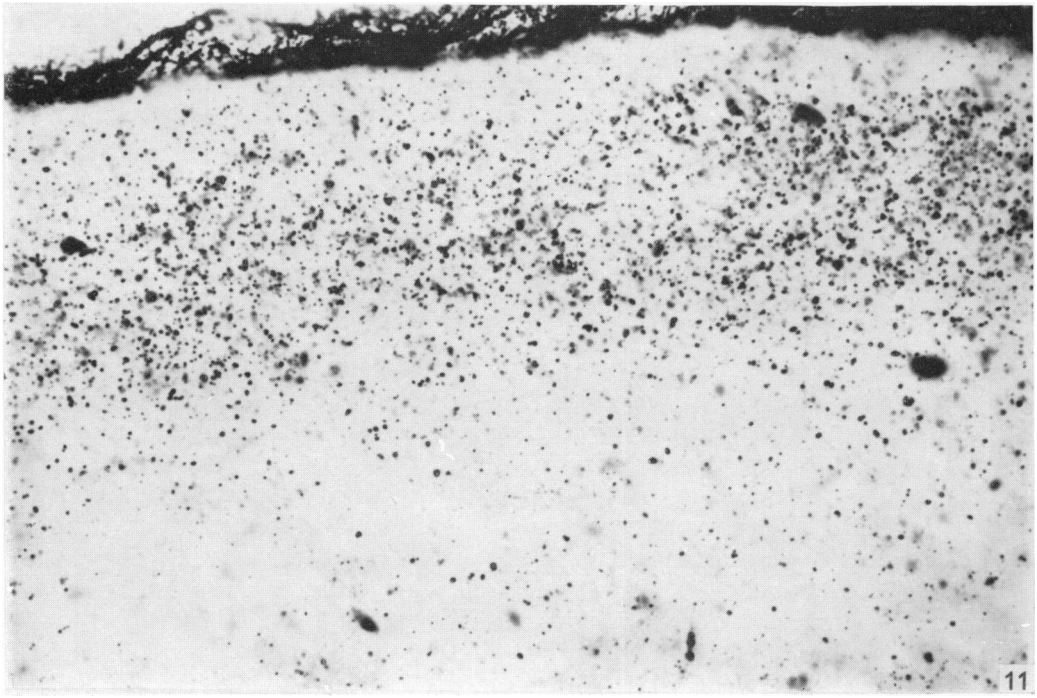

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A final point is that the terminal degeneration within the pyriform cortex itself following damage to this cortex is largely restricted to the deep half of the plexiform layer, so that its distribution is complementary to that seen after a lesion of the olfactory bulb. This confirms the earlier report by Heimer (1968).

Fibre pathway to the anterior hippocampus

In brains with lesions of the posterior pyriform cortex, such as R 699 (Fig. 8), an unexpected projection to the anterior rudiment of the hippocampus was found. Since this projection is not necessarily related to the olfactory system proper, it has been separated from the previous description and will be considered separately. As well as destroying a substantial area of cortex, the lesion in R 699 extended through the entire thickness of the cortex down to the white matter, although the amygdaloid nuclei were not damaged.

From the lesion, extensive fibre degeneration can be seen passing rostrally, first in the deepest layer of the pyriform cortex and then in the medial forebrain bundle; most of these fibres pass medially to the thalamus and hypothalamus (Powell *et al.* 1965) or continue rostrally to the anterior part of the pyriform cortex and the anterior olfactory nucleus (Heimer, 1968). However, at the level of the anterior part of the septum a distinct bundle of fibres can also be seen leaving the medial forebrain bundle to pass medially, deep to the olfactory tubercle, towards the diagonal band. But instead of passing into the septum in the diagonal band, the degenerating fibres pass forwards and then dorsally, within the cortex on the medial surface of the hemisphere immediately in front of the septum, to reach the anterior hippocampus; here they have a very circumscribed termination in the superficial plexiform layer, which is filled with the fine silver granules indicative of terminal degeneration (Figs. 12, 13).

Damage to the entire thickness of the pyriform cortex caudal to the level of the septum consistently results in at least partial degeneration of this pathway to the anterior hippocampus. The fibres do not arise generally from the whole of the pyriform cortex, because lesions of the anterior part of the pyriform cortex such as R 681 and R 685 (Fig. 7) have never been found to produce degeneration in the anterior hippocampus. This projection to the anterior hippocampus would therefore appear to arise either in the posterior part of the pyriform cortex or in regions caudal or medial to this cortex, and to pass forwards immediately deep to the posterior part of the pyriform cortex as far as the level of the septum or anterior amygdaloid area, where it joins the lateral component of the medial forebrain bundle.

In an attempt to determine whether these fibres originate posterior to the pyriform cortex, three large lesions were placed in the entorhinal region (Fig. 15). In R 822 most of the surface extent of the entorhinal cortex was damaged and in R 786,

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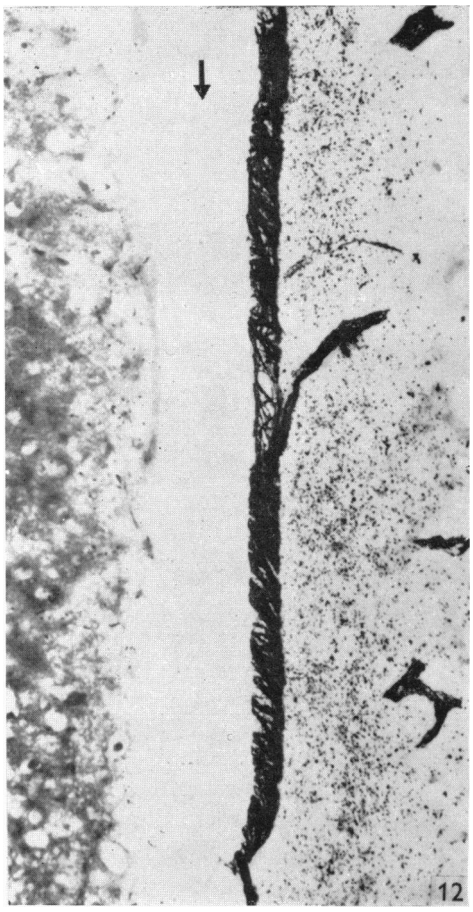
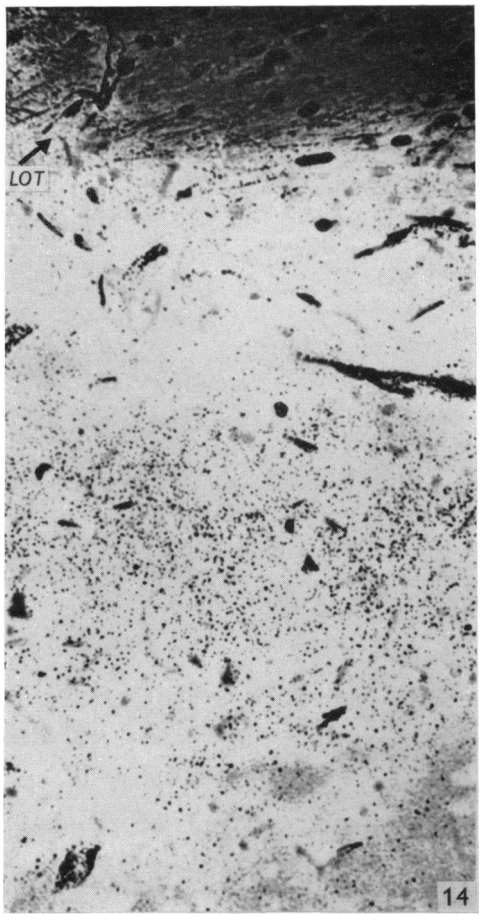
Figs. 9, 10. Photomicrographs to show the appearance of degenerating fibres in the lateral part of the stria medullaris of the operated side (Fig. 9) in experiment 699 as compared with the corresponding part of the stria on the normal side of the same section (Fig. 10). Fink–Heimer method. × 160.

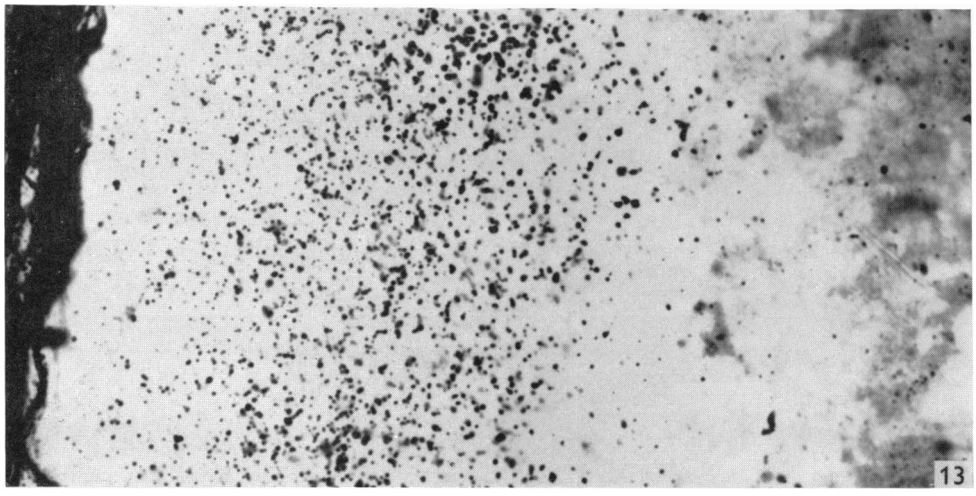
Fig. 11. Terminal degeneration in the superficial part of the molecular layer of the area of transitional cortex on the caudal surface of the hemisphere, between the pyriform cortex and the entorhinal area, after a lesion confined to the olfactory bulb. Fink–Heimer method. × 540.



12



14



13

although the lateral entorhinal area was principally damaged, the lesion extended into the white matter and should have interrupted any fibres passing laterally from the medial entorhinal area; experiment R 823 is another example of a deep lesion involving the dorsal parts of the lateral and medial entorhinal areas.

Although there is well-marked fibre and terminal degeneration in the hippocampus proper in these brains, no degeneration is present in the anterior hippocampal rudiment. In R 786, in which the postero-dorsal part of the hippocampus was damaged, there is severe degeneration in the septum adjacent to the anterior hippocampus, indicating that the absence of degeneration in the latter is not due to imperfect impregnation. From this experiment it may also be seen that the hippocampus itself does not project to the anterior rudiment, a conclusion which is in accord with other experiments in which the fimbria was almost completely interrupted.

These findings suggest that most of the entorhinal cortex does not contribute to the projection to the anterior hippocampus, but it should be noted that these experiments do not exclude an origin from more ventral parts of the entorhinal cortex where it adjoins the pyriform cortex, including that portion (described above) which receives a direct projection from the olfactory bulb.

Several brains with lesions in the baso-lateral nuclei of the amygdala are also available in which there is clear degeneration in the anterior hippocampus, but in all these experiments there is concomitant damage of the external capsule and the deepest part of the pyriform cortex.

DISCUSSION

As has been stated, this investigation was undertaken as an attempt to clarify certain discrepancies concerning the projection of the olfactory bulb, and in doing so observations have been made on other fibre connexions of the olfactory areas of the forebrain. It is convenient to discuss each of these points separately.

The entorhinal area

The present findings are in agreement with those of White (1965) and Heimer (1968) in the rat and of Scalia (1966) in the rabbit, in showing that the olfactory bulb projects to a region caudal to the pyriform cortex. However, these secondary olfactory fibres do not enter the entorhinal cortex as originally described by Cajal under the name 'spheno-occipital ganglion' or 'écorce temporal supérieure ou postérieure' (Cajal, 1911); after lesions of the olfactory bulb the degeneration stops sharply at the border of the spheno-occipital ganglion, filling the whole of Cajal's 'olfactory sphenoidal cortex'. Degeneration has also been found in this region after lesions of

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Fig. 12. The superficial plexiform layer of the anterior hippocampus of the normal and operated sides of a brain with a large lesion involving the entorhinal area; the arrow marks the mid-line. On the operated side there is fine terminal degeneration. Fink–Heimer method. × 240.

Fig. 13. The terminal degeneration in the same section of the anterior hippocampus at a higher magnification. × 830.

Fig. 14. The terminal degeneration in the ventral part of the anterior olfactory nucleus of the contralateral side due to the lesion in R 764. Note that the degeneration is restricted to the deep half of the plexiform layer. Fink–Heimer method. × 290.

the pyriform cortex, but as these lesions would have interrupted fibres from the olfactory bulb, and as the distribution of the degeneration is precisely the same as after lesions of the olfactory bulb, it cannot be determined whether there is an additional projection from the pyriform cortex.

Following Cajal's description of the spheno-occipital ganglion, this area was studied by several workers, including Brodmann (1909), Rose (1929, 1931) and Lorente de Nó (1933, 1934). Brodmann named the region the entorhinal cortex, or area 28, and subdivided it into 28*a* (lateral) and 28*b* (medial), while Rose divided

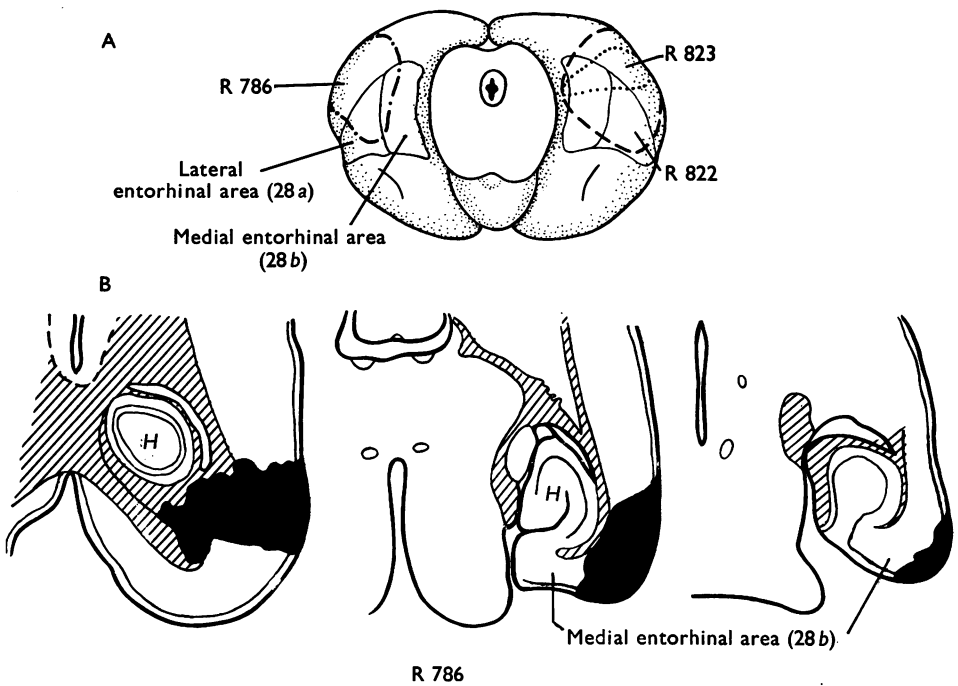

Fig. 15. A, The extent of the lesions in R 786 (–·–), R 822 (– – –) and R 823 (. . . . . .) in the entorhinal cortex, drawn on the caudal surface of the cerebral hemispheres of the rat. All these brains were cut horizontally, in order to see the entorhinal area more clearly. B, Tracings of three horizontal sections of R 786 to show the depth of the lesion.

it into a varying number of subfields, recognizing five and nine subdivisions respectively in such closely related animals as the mouse and the rabbit. The entorhinal area described by Rose also includes considerably more than the spheno-occipital ganglion; in the rabbit it would appear that only subdivisions e 8 and e 9 of Rose correspond to Cajal's 'ganglion' (see Scalia, 1966). With some modifications, Lorente de Nó accepts Brodmann's medio-lateral subdivision of the entorhinal cortex, but he rejects Rose's variable subclassification. Lorente de Nó appears to be dealing with the same cortical area as Cajal, although he also includes the perirhinal area (area 35) of Brodmann within the entorhinal cortex. White (1965) and Heimer (1968) indicate only that the olfactory bulb projects to the ventro-lateral part of the entorhinal area, but Scalia (1966) has tried to relate the distribution of the secondary olfactory fibres to the subdivisions made by Rose in the rabbit. Scalia found that

the olfactory fibres do not enter the spheno-occipital ganglion (e 8 and e 9), but also that they do not terminate in area e 7, which he identified as being outside the spheno-occipital ganglion. Rose did not describe the rat brain, and it is obviously very difficult to transfer his classifications from one animal to another, so no attempt has been made to correlate our results with his maps. It should be noted that Dennis & Kerr (1968), using evoked potential methods in the cat, have also concluded that the olfactory bulb projects beyond the pyriform cortex, to what they have termed the 'parahippocampal cortex'; this area corresponds very well with the similar area described by Scalia and ourselves.

The ventro-lateral part of the entorhinal region can therefore be recognized as distinct from Cajal's spheno-occipital ganglion on the basis of both architectonic structure and connexions. This ventro-lateral portion is transitional in structure between the pyriform cortex and the spheno-occipital ganglion, and it may be suggested that it should be considered as separate from the rest of the entorhinal area. Although it is well known that the entorhinal cortex is a major source of afferent fibres to the hippocampus, all the studies which have been done on this pathway have either been on the spheno-occipital ganglion itself or else have not taken into account possible differences within the entorhinal region, except for the medio-lateral differences which Cajal (1911) originally described (Lorente de Nó, 1934; Allen, 1948; Adey & Meyer, 1952; Blackstad, 1958; Raisman, Cowan & Powell, 1965). There is therefore no evidence in the literature that the hippocampus receives fibres from any part of the entorhinal area except for the spheno-occipital ganglion, and it is possible that the 'transitional' cortex may have different efferent as well as afferent connexions.

It may also be noted that there is a similar cortical area between the neocortex and the entorhinal area, Brodmann's area 35 or perirhinal cortex; Brodmann (1909) described this region, which lies in the rhinal sulcus, as being transitional in structure between the neocortex and the entorhinal cortex. Although Lorente de Nó included area 35 within the entorhinal cortex, he stated that this transitional area could be distinguished. It has been shown in the cat (Cragg, 1965; Diamond, Jones & Powell, 1968) and the monkey (Whitlock & Nauta, 1956; Jones & Powell, 1970) that the neocortical projection to the entorhinal region is into the banks of the rhinal sulcus. (Cragg also shows a sparse projection into the deepest layers of the entorhinal cortex proper). In most of these studies the site of termination of the fibres from the neocortex was described as being the lateral part of the entorhinal area, but it has been suggested (Jones & Powell, 1970) that these fibres terminate in the perirhinal cortex, area 35. To determine whether this projection is also present in the rat, lesions were placed in the neocortex of two rats, just anterior to the entorhinal area. In these brains there is heavy terminal degeneration in area 35; in the superficial layers this degeneration stops abruptly at the edge of the entorhinal cortex, but in the deep layers a little fragmentation spreads into the deepest layers of the entorhinal area proper.

It is therefore suggested that there is a region of transitional cortex around the entorhinal area proper, and that this can be subdivided into two parts, the first lying between the entorhinal area and the pyriform cortex, and the second between the entorhinal area and the neocortex. It would also appear that the major projec-

tions to the entorhinal cortex from the olfactory system and from the neocortex are through these transitional zones rather than direct. It may justifiably be argued that this suggestion exaggerates the importance of such a 'transition zone', but it should be noted that a similar zone of transition between the somatic sensory and motor areas of the neocortex, area 3*a*, has been shown to be a specific functional subdivision in being the cortical projection area of group 1 muscle afferents (Oscarsson & Rosén, 1966; Phillips, Powell & Wiesendanger, 1970).

The olfactory tubercle

Degeneration of fibres and terminals has been found throughout the entire extent of the olfactory tubercle after lesions which damage the ventral part of the olfactory bulb, or after lesions which destroy all of the bulb and extend into the anterior olfactory nucleus. On the other hand, after lesions restricted to the dorsal part of the olfactory bulb, degeneration has been found only in the antero-lateral portion of the olfactory tubercle. This evidence suggests that there may be a gross topographical organization in the projection of the olfactory bulb on to the tubercle. While it is difficult to explain all the discrepancies in the literature on this basis, an important contributing factor in the failure of some previous investigators to find an olfactory projection to the postero-medial part of the olfactory tubercle may have been that their lesions were predominantly in the dorsal part of the olfactory bulb, and therefore produced degeneration only in the antero-lateral part of the olfactory tubercle. Two authors have previously reported a projection to the entire olfactory tubercle, and it is worth noting that the lesions which White (1965) indicated as producing definite degeneration in the postero-medial part of the tubercle caused extensive damage in the olfactory bulb, and also extended into the anterior olfactory nucleus; the lesions made by Heimer (1968) in the olfactory bulb apparently involved a substantial proportion of both the dorsal and the ventral parts of the bulb.

Since the experiments of Adrian (1950), which provided physiological evidence for a certain degree of topical localization within the olfactory bulb, and those of Le Gros Clark (1951, 1957), which produced anatomical evidence for some topographical organization in the projection of the olfactory mucosa on the olfactory bulb, there has been speculation as to whether there is a topographical organization in the more central olfactory connections. As Le Gros Clark noted, if the analysis of olfactory stimuli depends on receptors of differential sensitivity, and if these receptors project in an organized manner to different groups of mitral cells within the olfactory bulb, 'it seems reasonable to suppose that the segregation must be maintained as far as the olfactory centres in the brain which provide the ultimate basis for the conscious discrimination of smells' (Le Gros Clark, 1957). In the case of the major site of termination of olfactory bulb fibres, the pyriform cortex, no such organization has yet been demonstrated. White (1965) and Lohman & Mentink (1969) report that small lesions in the olfactory bulb produce degeneration throughout the whole of the 'olfactory cortex', with only the density of degeneration varying with the size of the lesion; if by 'olfactory cortex' they mean the pyriform cortex, then their results are in agreement with those presented here, for no difference was found in the degeneration in the pyriform cortex after lesions in the dorsal or the ventral parts of the olfactory bulb. Nevertheless, the finding of Lohman & Mentink (1969)

that only the medial part of the olfactory bulb projects to the medial part of the pars dorsalis of the anterior olfactory nucleus, taken with the present evidence that only the ventral part of the bulb projects to the postero-medial portion of the olfactory tubercle, indicates that there may be some topographical organization in the projection upon the anterior olfactory nucleus and the olfactory tubercle. The evidence is as yet insufficient to support more than a suggestion that such an organization exists, but if it is in fact present, it may reflect an important functional difference between the pyriform cortex on the one hand, and the olfactory tubercle and the anterior olfactory nucleus on the other.

The stria medullaris

In addition to the projection from the pyriform cortex to the medio-dorsal thalamic nucleus and the lateral habenular nucleus through the thalamic radiation (Powell *et al.* 1965), a fibre pathway to the lateral habenular nucleus through the stria medullaris has now been found, and it is probable that the number of fibres in this pathway from the entire pyriform cortex is quite considerable.

This finding that a substantial component of the stria medullaris arises in the pyriform cortex indicates that this fibre bundle and the habenular nuclei are more closely related to the olfactory system in the mammal than had previously been thought. There are two other major components of the stria medullaris: the first is a commissural projection from the nucleus of the horizontal limb of the diagonal band of one side to the same nucleus of the opposite side through the habenular commissure (Price & Powell, 1970*b*); this nucleus has been shown to be the nucleus of origin of the centrifugal fibres to the olfactory bulb (Price & Powell, 1970*a*). The second is a projection in the medial part of the stria medullaris from the septum to the medial habenular nucleus (Nauta, 1956, 1958; Valenstein & Nauta, 1959, Cragg, 1961; Raisman, 1966). Although there may be other, smaller components, it would appear that the bulk of the stria medullaris is made up of these three projections.

This strong connexion of the stria medullaris with the olfactory system is even more definite in reptiles. Experimental investigations in the lizard (Gamble, 1952; Heimer, 1969) and the turtle (Gamble, 1956) have shown that fibres from the olfactory bulb run backwards through the lateral part of the diagonal band and into the stria medullaris, cross in the habenular commissure, and pass forwards again through the stria medullaris and diagonal band to reach the olfactory bulb of the opposite side. Studies on the normal brain of the lizard have also demonstrated fibres from the lateral cortex (probably analogous to the pyriform cortex) entering the lateral part of the stria medullaris (Goldby, 1934).

The anterior hippocampus

Although the early studies on the normal brains of submammalian animals almost invariably indicated that the olfactory bulb projected to the primordium hippocampi (Nieuwenhuys, 1967), more recent experimental work has failed to confirm this; in the nurse shark (Ebbeson & Heimer, 1969), the lizard (Gamble, 1952; Heimer, 1969) and the turtle (Gamble, 1956) the only pallial structure found to receive secondary olfactory fibres is the lateral cortex. It is not surprising therefore that we have

been unable to confirm the report of Scalia (1966) that the olfactory bulb projects to the anterior rudiment of the hippocampus.

However, this apparent contradiction may be due to differences in terminology. The antero-ventral portion of the anterior hippocampus is directly contiguous with the medial part of the anterior olfactory nucleus, and in some of the older accounts both these structures are included in the 'anterior hippocampus'. Scalia found an olfactory projection only to the antero-ventral portion of the 'anterior hippocampus' and from his figures it would appear that he is referring to the area which we, following Lohman (1963), have termed the pars medialis of the anterior olfactory nucleus.

However, a fibre pathway to this nucleus has been found from more caudal portions of the rhinencephalon. The fibres degenerate after lesions of the posterior pyriform cortex and amygdala and terminate specifically in the plexiform layer of the anterior hippocampus. Degenerating fibres in this area after similar lesions were described by Cowan, Raisman & Powell (1965), but their termination was not identified. The specificity of this pathway, together with the almost complete lack of knowledge of other afferent connexions of the anterior hippocampus, gives some interest to this finding, but the implications are difficult to assess without more exact knowledge of its origin. It was originally expected that the fibres would arise in some part of the entorhinal cortex, but as experiments with lesions of the entorhinal area have so far failed to substantiate this hypothesis, no firm statement can be made on their origin. The significance of this discovery of an afferent pathway to the anterior hippocampus from the more caudal parts of the rhinencephalon is possibly to be found in the recent report of a projection from the anterior hippocampus to the paraventricular nucleus of the hypothalamus (Woods, Holland & Powell, 1969). It would thus suggest that, in addition to the well-established pathway from the entorhinal area through the hippocampus and fimbrial system to the hypothalamus, there is a route from either the entorhinal area or adjoining regions to the hypothalamus through the anterior hippocampus.

SUMMARY

1. In order to investigate certain discrepancies in the literature concerning the olfactory pathways, lesions were placed in several parts of the olfactory system, and the brains studied for the resultant axonal degeneration, using various reduced silver methods.

2. The olfactory bulb has been found to send fibres to the cortex caudal to the pyriform cortex, into a part of what has previously been considered to be the entorhinal region; the olfactory fibres do not, however, enter the spheno-occipital ganglion of Cajal (1911). Lesions of the pyriform cortex necessarily interrupt fibres from the olfactory bulb, and such lesions produce the same pattern of degeneration in the region as do lesions of the bulb. It is suggested that the area which receives direct olfactory fibres is a transitional zone between the pyriform cortex and the 'true' entorhinal cortex (Cajal's spheno-occipital ganglion) and that it corresponds to a similar transition zone, area 35 or the perirhinal cortex, between the neocortex and the entorhinal cortex.

3. Lesions restricted to the dorsal part of the olfactory bulb produce degeneration only in the antero-lateral part of the olfactory tubercle, but lesions which damage the ventral part of the bulb result in degeneration throughout the olfactory tubercle. The entire extent of the olfactory tubercle thus receives a direct projection from the olfactory bulb, and this projection may have some degree of topographical organization.

4. The fibres from the anterior olfactory nucleus of one side which cross in the anterior commissure to end in the anterior olfactory nucleus of the other side have been found to terminate predominantly in the deep half of the superficial plexiform layer of that structure.

5. The pyriform cortex sends a substantial projection into the stria medullaris, and these fibres terminate in the lateral habenular nucleus.

6. The anterior rudiment of the hippocampus has been found to receive fibres from the caudal parts of the rhinencephalon but the precise origin of this pathway has not been determined. No fibres from the olfactory bulb have been found to terminate in the anterior hippocampus.

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